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Serological Evidence of SV40 Infections in HIV-Infected and HIV-Negative Adults

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SV40 is a simian polyomavirus that was a contaminant of some viral vaccines administered to people between 1955 and 1962. SV40 DNA has recently been found associated with several types of human tumors, suggesting that the virus is present in humans. We examined sera from patients infected with human immunodeficiency virus type 1 (HIV-1) as well as from HIV-1–negative controls to determine the prevalence of SV40 neutralizing antibodies using a specific plaque reduction assay. We found that 16.1% of HIV-infected patients (n = 236) were seropositive for SV40, as compared to 12.0% of HIV-negative control volunteers (n = 108) and 11.1% of HIV-negative patients (n = 72). These differences were not statistically significant. As individuals born between 1941 and 1962 had the highest chance of having received SV40-contaminated poliovaccines, we analyzed SV40 seropositivity rates based on year of birth. SV40 antibody rates for HIV-infected patients born before 1941, between 1941 and 1962, and after 1962 were 17.1%, 16.3%, and 11.8%, respectively. For the HIV-negative subjects, the rates were 12.5%, 12.0%, and 9.7%, respectively. There was no correlation between SV40 seropositivity and either the stage of disease in HIV-infected patients or the race/ethnicity. Also, there was no correlation between the presence of SV40 neutralizing antibody and the titer of neutralizing antibody to human polyomavirus BKV. The SV40 seropositivity rates in the patients born between 1941 and 1962 may be explained by the likelihood of those individuals having received SV40-contaminated vaccines, but the detection of SV40 neutralizing antibody in individuals born after 1962 (with no risk of having received contaminated vaccines) is significant. Although cross-reactive antibodies might theoretically contribute to the observed reactivities, these results suggest that SV40 neutralizing antibodies are present in certain individuals and raise the possibility that SV40 continues to infect humans long after vaccines were freed from contamination. ***J. Med. Virol. 54:276–284, 1998.*** © 1998 Wiley-Liss, Inc.

KEY WORDS: SV40; human infections by SV40; neutralizing antibodies to SV40; polyomaviruses; SV40 and HIV-infected adults

INTRODUCTION

SV40, a simian polyomavirus, was discovered in 1960 as a contaminant of viral vaccines and of monkey kidney cells used to produce the vaccines [Sweet and Hilleman, 1960]. The contaminated vaccines were used between 1955 and 1962, during which time they were administered to millions of vaccinees [Shah and Nathanson, 1976], providing a documented source of human exposure to SV40. The virus is widely recognized as having potent transforming potential in experimental systems [Butel, 1994; Conzen and Cole, 1994; Cole, 1996]. Recently, SV40 DNA has been found in association with human brain tumors [Bergsagel et al., 1992; Lednicky et al., 1995; Martini et al., 1996], mesotheliomas [Carbone et al., 1994, 1997; Pepper et al., 1996], and osteosarcomas [Carbone et al., 1996; Lednicky et al., 1997], although other investigators have failed to detect SV40 sequences in human mesothelioma [Strickler et al., 1996]. Detection of four separated regions of the viral genome coupled with extensive sequence analyses on some of the specimens established conclusively that authentic SV40 was present in those virus-positive tumors [Lednicky et al., 1995, 1997; Stewart et al., 1998]. Although an etiological role for the virus in the DNA-positive tumors has not been proved, the findings raise the possibility that SV40 may be a factor in the development of some

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TABLE I. Age Distribution of Study Population

Subjects	Number	Mean age[a]	Range[a]	Median age[a]
HIV-infected patients	236	46	25–75	45
HIV-negative healthy volunteers	108	42	26–66	41
HIV-negative patients[b]	72	50	25–78	48

[a]In years.
[b]HIV-negative patients were non-immunosuppressed, with a variety of chronic underlying conditions.

human cancers. Many of the virus-positive tumors occurred in patients too young to have received any contaminated vaccines [Bergsagel et al., 1992; Lednicky et al., 1995, 1997; Carbone et al., 1996; Martini et al., 1996], suggesting that SV40 must be present in the human population. Indeed, in studies carried out about 20 years ago, sera from individuals who had never received contaminated vaccines, including those born after 1962, displayed a low frequency of neutralizing antibody to SV40 (3–13%), suggesting other sources of exposure to SV40 or to a closely related virus [Shah et al., 1971, 1972; Shah, 1972; Zimmermann et al., 1983; Geissler et al., 1985].

The potential involvement of SV40 in human carcinogenesis emphasizes the need to know the prevalence of SV40 infections in humans in order to identify individuals who may be at risk of SV40-associated disease and to develop preventative approaches. We performed serological assays using a specific plaque reduction test for SV40 neutralizing antibodies to determine estimates of the current frequency of SV40 infections in humans. Because of the association of human disease caused by the human polyomaviruses with immunocompromised states [Berger and Concha, 1995; Shah, 1996] and because SV40 has been associated with the development of brain lesions and progressive multifocal leukoencephalopathy in monkeys immunocompromised due to infection with simian immunodeficiency virus [Holmberg et al., 1977; Horvath et al., 1992; Ilyinskii et al., 1992; Lednicky et al., 1998], we compared sera from HIV-1–infected and non-HIV-1–infected adults for SV40 seropositivity. We found SV40 neutralizing antibody in 11 to 16% of these populations, but HIV infection did not influence SV40 seropositivity.

MATERIALS AND METHODS

Study Population

Two hundred and thirty-six HIV-1–infected men (mean age, 46 years), enrolled in a pneumococcal vaccine trial at the Veterans Administration Medical Center (VAMC) hospital in Houston, Texas, and from whom residual sera were available for study, were evaluated. Control groups were 72 HIV-1–negative patients (mean age, 50 years) followed at the VAMC for a variety of conditions and 108 HIV-1–negative non-veteran healthy volunteers (mean age, 42 years) (Table I).

Serum Samples

Sera from the patients and volunteers were collected between 1990 and 1995 and were stored at −70°C at the VAMC hospital. For this study, aliquots were removed, heat-inactivated at 56°C for 30 min, transported on ice to Baylor College of Medicine, and stored at −70°C.

Hyperimmune rabbit serum prepared against purified SV40 was described previously [Butel et al., 1984]. Hyperimmune rabbit sera raised against purified BKV were kindly provided by T. Flaegstad and K.V. Shah.

Cells and Virus

Monkey kidney TC-7 cells were used for SV40 plaque assays and were subcultured in enriched Eagle medium [Noonan et al., 1976] containing 5% fetal bovine serum (FBS; Life Technologies, Grand Island, NY). Vero cells were used for BKV immunohistochemical assays and were passaged in 199 medium supplemented with 5% FBS and glutamine.

The Baylor strain of SV40 was used [Melnick and Stinebaugh, 1962; Lednicky and Butel, 1997; Stewart et al., 1998]. Purified BKV, strain Dunlop, was a gift from T. Flaegstad [Flaegstad and Traavik, 1985].

SV40 Neutralization Assay

A plaque reduction assay was used to detect SV40 serological reactivity, as neutralization of virus infectivity is the most specific measurement of viral antibodies [Hsiung et al., 1994]. SV40 plaque assays were performed as previously described [Noonan and Butel, 1978] using TC-7 cells. Heat-inactivated serum samples were diluted in Tris-buffered saline (TBS; pH 7.4) and mixed with equal volumes of SV40 diluted to contain 50 to 100 pfu per 0.1 ml. Each assay included the following controls: virus only (virus mixed with TBS), normal serum control (virus mixed with normal rabbit or human serum lacking SV40 antibodies), positive serum control (virus mixed with hyperimmune rabbit serum with high neutralizing activity against SV40) [Butel et al., 1984], and cell controls (TBS only). All the samples were incubated at 37°C for 30 min. TC-7 cell monolayers in 60-mm^2 tissue culture plates were then inoculated with 0.2 ml of virus-serum mixture per culture and inocula were adsorbed for 2 hr at 37°C in a humidified 5% CO_2 incubator. Cell monolayers were then overlaid with a mixture of agar and enriched Eagle medium; a second overlay was applied on day 7; and a third overlay containing neutral red was applied on day 11. Plaques were counted on day 15. Each sample was tested in triplicate. Initial tests were carried out using final serum dilutions of 1:20, and positive samples (those that reduced the number of plaques by $\geq$50% compared to the virus-only control) were titered in repeat experiments to determine the endpoint neutralization titers.

BKV Neutralization Assay

A BKV immunoperoxidase neutralization assay was performed using Vero cell monolayers in 96-well mi-

TABLE II. SV40 Neutralizing Antibody Prevalence in HIV-Infected and HIV-Negative Adults

Group	Number of sera tested	Number with antibodies (%)	Antibody titers of positive sera			
			1:20	1:40	1:160	⩾1:320
HIV-infected patients	236	38 (16.1)	16	16	3	3
HIV-negative volunteers	108	13 (12.0)	6	5	1	1
HIV-negative patients	72	8 (11.1)	2	6	0	0
All HIV negative[a]	180	21 (11.7)	8	11	1	1
Total	416	59 (14.2)	24	27	4	4

[a]The sum of the 2 HIV-negative groups.

crotiter plates as previously described [Flaegstad et al., 1986a], with some modifications. Two days after cell seeding, the medium was removed and the cells were infected with BKV virus-serum mixtures, prepared, and incubated as described above. The dilution of BKV used induced 50 to 100 antigen-positive cells per 0.1 ml. After 1 to 2 hr of adsorption at 37°C, inocula were removed, the wells were washed with media containing 1% FBS, and the cells were overlaid with media containing 1% FBS and 1.5% carboxymethylcellulose. Five days postinfection the overlay was removed and the monolayers were fixed in 80% acetone in phosphate buffered saline (pH 7.4). The cells were incubated with rabbit anti-BKV antibody (a gift from T. Flaegstad). Positive cells were stained using a Vectastain ABC kit (Vector Laboratories, Inc., Burlington, CA), and antigen-positive cells were counted under a microscope.

Statistical Analysis

Statistical significance was determined by chi-square analysis.

RESULTS

Both HIV-Infected and HIV-Negative Adults Harbor Serum Neutralizing Antibodies to SV40

Sera from 416 adult men were examined for neutralizing antibodies to SV40. The results are shown in Table II. The plaque reduction assays revealed that 16.1% of HIV-infected patients were positive for SV40 neutralizing antibodies, as compared to 12.0% and 11.1% of the two HIV-negative groups. There was no significant difference between the SV40 seropositivities of the HIV-infected and HIV-negative populations. The neutralizing antibody titers were relatively low in most individuals (1:20 or 1:40), although some titers reached levels ⩾1:320. These titers are comparable to those reported in other studies of SV40 antibody responses in humans [Shah et al., 1971, 1972; Horváth, 1972; Shah, 1972; Brown and Morris, 1976].

Age and SV40 Seropositivity

Many individuals in the United States were exposed to poliovaccine potentially contaminated with SV40. About 98 million people were immunized between 1955 and 1962 with inactivated poliovaccines contaminated to varying extents with SV40; some vaccine lots contained residual live (not inactivated) SV40. In addition, more than 100,000 military inductees received SV40-contaminated adenovirus vaccines between 1955 and 1961. All vaccines produced after mid-1961 were required to be free from SV40, although some contaminated vaccine might have been stored and used in 1962. It is not possible to know with certainty who received contaminated poliovaccine, but educated estimates have been made [Shah and Nathanson, 1976]: those born between 1941 and 1961 had the highest risk of exposure, those born before 1941 had a low risk of exposure, and those born after 1962 would have had no risk of exposure to SV40-contaminated vaccine. Therefore we grouped the HIV-infected and HIV-negative individuals by year of birth into three groups, (1) born before 1941, (2) born between 1941 and 1962, and (3) born after 1962, and analyzed the seropositivity rates of the different groups (Table III). Both of the older age groups (pre-1941 and 1941–1962) averaged about 15% positive for SV40 neutralizing antibody. There is the possibility these individuals received SV40-contaminated vaccine. Although the civilian pre-1941 age group had a lower predicted risk of exposure than the 1941 to 1962 group, it is possible that the veterans born before 1941 were exposed to SV40-contaminated viral vaccines while in military service. The group born after 1962 displayed an SV40 seropositivity rate of about 10%, even though those individuals had no risk of exposure to contaminated vaccine. This indicates that there are sources of human infection by SV40 other than the contaminated virus vaccines used between 1955 and 1961. From the age comparison it appeared that HIV-infected individuals had slightly higher rates of SV40 antibodies than the HIV-negative ones, but there were no significant differences among the groups.

Race/Ethnicity and SV40 Seropositivity

Information on race/ethnicity background was available for the HIV-infected patients and the HIV-negative volunteers. Whites and hispanics who were HIV-infected had higher rates of SV40 seropositivity (16.4% and 26.3%, respectively) than did African-Americans (13.5%) (Table IV). In all cases the HIV-infected groups had higher rates of antibody positivity to SV40 than did the equivalent HIV-negative ethnic groups, but the differences were without statistical significance. Thus race/ethnicity did not affect SV40 seropositivity in this study population.

TABLE III. SV40 Seropositivity in HIV-Infected and HIV-Negative Adults According to Age Group

Group	Year of birth			All ages
	Pre-1941	1941–1962	Post-1962	
HIV-infected	6/35 (17.1)[a,b]	30/184 (16.3)	2/17 (11.8)	38/236 (16.1)
HIV-negative volunteers	2/11 (18.2)	8/71 (11.3)	3/26 (11.5)	13/108 (12.0)
HIV-negative patients	2/21 (9.5)	6/46 (13.0)	0/5	8/72 (11.1)
All HIV negative[c]	4/32 (12.5)	14/117 (12.0)	3/31 (9.7)	21/180 (11.7)
All groups	10/67 (14.9)	44/301 (14.6)	5/48 (10.4)	59/416 (14.2)

[a]SV40 seropositivity was determined by plaque reduction assays. Numerator, number of sera positive; denominator, number of sera tested.
[b]Percentage positive is given in parentheses.
[c]This group is the sum of the 2 HIV-negative groups.

TABLE IV. SV40 Seropositivity in HIV-Infected and HIV-Negative Adults According to Race/Ethnicity Background

Group	Race/Ethnicity	Year of birth			All ages
		Pre-1941	1941–1962	Post-1962	
HIV-infected	White	4/23 (17.3)[a,b]	18/105 (17.1)	0/6 (0)	22/134 (16.4)
	African-American	2/10 (20.0)	7/57 (12.2)	1/7 (14.2)	10/74 (13.5)
	Hispanic	0/2 (0)	4/15 (26.6)	1/2 (50.0)	5/19 (26.3)
	Other/unknown	0/0 (0)	1/7 (14.2)	0/2 (0)	1/9 (11.1)
	Total	6/35 (17.1)	30/184 (16.3)	2/17 (11.7)	38/236 (16.1)
HIV-negative volunteers	White	2/7 (28.5)	4/31 (12.9)	2/19 (10.5)	8/57 (14.0)
	African-American	0/3 (0)	4/28 (14.2)	0/4 (0)	4/35 (11.4)
	Hispanic	0/0 (0)	0/12 (0)	0/1 (0)	0/13 (0)
	Other/unknown	0/1 (0)	0/0 (0)	1/2 (50.0)	1/3 (33.3)
	Total	2/11 (18.2)	8/71 (11.3)	3/26 (11.5)	13/108 (12.0)

[a]See footnote to Table III.
[b]Percentages are given in parentheses.

Disease Status of HIV-1–Infected Patients and SV40 Seropositivity

We next analyzed the HIV-1–infected subjects for possible correlation of SV40 seropositivity with whether they were asymptomatic or had symptomatic disease (Table V). Fourteen percent of the HIV-infected asymptomatic patients had SV40 neutralizing antibodies, compared to ≈19% of the symptomatic patients. Again, the difference was not statistically significant. This indicated that disease status in HIV-1–infected persons was not associated with a different frequency of SV40 seropositivity.

Lack of Correlation of SV40 Seropositivity with BKV Antibody Titers

The potential cross-reactivity of polyomavirus antigens was considered for possible influences on the detection of SV40 antibodies (see Discussion). We addressed the question of whether the SV40 antibody-positive individuals might be those with the highest BKV antibody titers and found that there was no such correlation. To do this, we performed BKV immunoperoxidase neutralization assays (see Materials and Methods). We compared anti-BKV and anti-SV40 serum titers in 10 HIV-negative persons and 12 HIV-infected patients (Fig. 1). All 22 adults were seropositive for anti-BKV antibodies. Six of 10 HIV-negative and 6 of 12 HIV-infected individuals had serum titers of ⩾1:20 to SV40. However, higher anti-BKV antibody titers did not correlate with SV40 seropositivity. Five of 8 persons with anti-BKV titers of 1:1600 were seronegative for antibodies to SV40, whereas others with anti-BKV titers as low as 1:100 or 1:200 contained SV40 neutralizing antibodies. We noted that 11 of 12 HIV-infected patients had anti-BKV titers ⩾1:800, as compared to only 3 of 10 HIV-negative persons ($P < 0.01$; Fisher's two-tailed test). Thus, higher anti-BKV titers did correlate with HIV infection in this group of individuals. As further reassurance of the specificity of the SV40 assay, 2 different hyperimmune rabbit sera having high neutralizing titers against BKV were tested by us, and neither had neutralizing activity at a low dilution against SV40 (data not shown). These data suggest that the SV40 neutralizing activity detected was not due to cross-reactive BKV antibodies.

DISCUSSION

SV40 DNA has been found associated with some human brain tumors [Bergsagel et al., 1992; Lednicky et al., 1995; Martini et al., 1996], osteosarcomas [Carbone et al., 1996; Lednicky et al., 1997], mesotheliomas [Carbone et al., 1994, 1997; Pepper et al., 1996], and normal human tissues [Woloschak et al., 1995; Martini et al., 1996]. However, these observations are somewhat controversial, as others have failed to detect viral sequences in mesotheliomas [Strickler et al., 1996]. Molecular studies have established conclusively that authentic SV40 sequences were present in some of the tumors. Four different regions of the SV40 genome were amplified and sequenced from several brain tu-

TABLE V. SV40 Seropositivity in HIV-Infected Adults According to Stage of Disease

Stage of disease	Number of patients	Year of birth			All ages
		Pre-1941	1941–1962	Post-1962	
Asymptomatic	139	4/20 (20.0)[a,b]	16/111 (14.4)	0/8 (0.0)	20/139 (14.4)
Symptomatic	97	2/15 (13.3)	14/73 (19.2)	2/9 (22.2)	18/97 (18.5)

[a]See footnote to Table III.
[b]Percentages are given in parentheses.

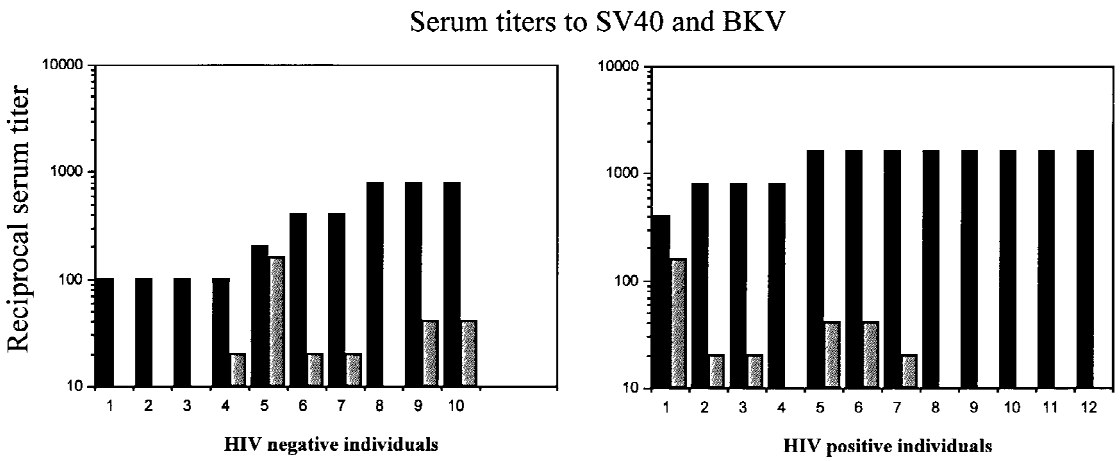

Fig. 1. SV40 and BKV antibody titers in groups of HIV-infected and HIV-negative individuals. Anti-BKV serum neutralizing titers were determined by an immunoperoxidase assay and anti-SV40 serum neutralizing titers were determined by a plaque reduction assay (see Materials and Methods). The reciprocal of the anti-BKV neutralizing antibody titer is shown by the filled bars and that of the anti-SV40 neutralizing antibody by the hatched bars. Individual patient numbers are indicated at the bottom.

mors and bone tumors, indicating the presence of SV40 rather than a new or hybrid virus bearing only limited similarity to SV40 [Lednicky et al., 1995, 1997]. Sequence differences between the tumor-associated DNAs and laboratory strains of SV40 ruled out the possibility of laboratory contamination of those tumor samples. The viral DNAs found in tumors had a single 72-bp element in the regulatory region, whereas most laboratory-adapted strains have duplications in that region [Stewart et al., 1998]. Differences were also found in the variable domain at the C-terminus of the SV40 T-antigen gene. Many of the tumor-associated viral sequences were unique in this region, suggesting that multiple strains of SV40 can be associated with human tumors. However, no sequences identified human-specific or tumor-type–specific strain associations, suggesting that SV40 has a relatively broad host range [Stewart et al., 1998]. Furthermore, an infectious isolate was recovered from a choroid plexus carcinoma [Lednicky et al., 1995] and proved to be authentic SV40 by total sequence analysis of the viral genome [Stewart et al., 1998]. These findings prompted the study described here to investigate the current frequency of human infections by SV40.

Serological studies carried out about two decades ago provide a baseline for this study. Laboratory workers who handled primary monkey cell lines and monkeys or SV40 had a prevalence of antibodies to SV40 in 41 to 55% [Horváth, 1972; Zimmermann et al., 1983]. It is well documented that SV40-contaminated poliovirus vaccines were widely administered to people in the late 1950s and early 1960s [reviewed by Shah and Nathanson, 1976]. Neutralizing antibodies to SV40 were detected in 19.8% of sera of a group of 141 Maryland children born between 1955 and 1957 who were at high risk of having received SV40-contaminated poliovaccine [Shah et al., 1972]. Seroconversions to SV40 following exposure to contaminated vaccines have been documented; the antibody responses were generally low-titered [Morris et al., 1961; Brown and Morris, 1972; Shah, 1972]. However, a low prevalence of serum antibodies to SV40 (3–13%) in people not exposed to the contaminated vaccines indicated other sources of exposure to SV40 or to a related virus [Shah et al., 1971, 1972; Shah, 1972; Zimmermann et al., 1983; Geissler et al., 1985].

We found that 16% of HIV-1–infected men and 12% of HIV-1–negative men were seropositive for SV40 neutralizing antibodies by plaque reduction assay (Tables II, III). These values are somewhat lower than the seroprevalence (≈20%) of children probably exposed to SV40-contaminated vaccines [Shah et al., 1972]. One difference is that the sera we analyzed were collected at least 25 years later than were the children's sera in the former study, and we do not know the longevity of antibodies induced by the contaminated vaccines. The SV40-contaminated vaccines used between 1955 and 1961 were given widely to people under 20 years of age [Shah and Nathanson, 1976], so that might explain the antibody detected in the people in our study population

born between 1941 and 1962. We also found a substantial number of SV40 seropositives in people born before 1941. It is less likely that SV40 antibodies in that group can be explained by vaccination, as individuals that age were at a relatively lower risk of exposure to contaminated poliovaccine. However, it is possible that older veterans in this study may have been vaccinated with SV40-contaminated vaccines during military service. We do not know how many of the volunteers in this study may have served in the military, and their seropositivity rate in the pre-1941 group was higher than that of the HIV-negative veteran patients (Table III). Alternative possible explanations for persisting serological reactivity include spread within a family of virus from vaccinated children to older individuals, infections/reinfections with SV40 from the environment, and reactivation and replication of a latent SV40 infection.

Notably, individuals born after 1962, with no risk of exposure to contaminated vaccines, had ≈10% positive rate for SV40 neutralizing antibody. This indicates that there must exist an alternative source of human infection by SV40. Whereas there are no lower mammals or arthropods known to serve as reservoirs of infection, other humans may be that source. Whether the use of contaminated vaccines introduced the virus as a new infection into the human population or merely broadened a preexisting infection is not known. The seropositivity rate of the post-1962 group (≈10%) is higher than that reported two decades ago [Shah, 1972; Shah et al., 1972; Brown et al., 1975] for persons not exposed to vaccines (2–5%). Whether this reflects a higher frequency of human infection by SV40 20 years later or an anomaly of sample size (n = 48) is not known and requires additional study. It is closer to the 13% of sera from a cohort born after 1962 found to contain SV40 antibodies using an ELISA test [Zimmermann et al., 1983].

We considered the possible complications of infections by the human polyomaviruses BKV and JCV on this study. These two human viruses infect children at an early age [Padgett and Walker, 1973, 1983; Flaegstad et al., 1986b; Frisque, 1994; Shah, 1996]. BKV antibodies are present in about 50% of children aged 4 years and in nearly all children by age 10 years, and are maintained in 70 to 80% of adults. JCV antibodies reach a prevalence of 50% of children by age 10 years and are found in about 75% of adults. Transmission of BKV and JCV occurs by unknown routes, but respiratory secretions and urine are believed to be important. It has been shown, using nucleotide variations to track JCV strains, that parent-to-child transmission is common, accounting for about 50% of JCV transmission [Kunitake et al., 1995]. Both viruses establish in the kidney in a persistent or latent form and may also be latent in B lymphocytes [Tornatore et al., 1992; Dörries et al., 1994; Azzi et al., 1996; Monaco et al., 1996]. JCV may also establish latent infections in the brain [Mori et al., 1991; White et al., 1992; Ferrante et al., 1995; Vago et al., 1996]. Immunocompetent individuals excrete low levels of virus in the urine. Estimates of frequencies of shedding by normal persons have averaged about 20% for both viruses, although estimates have ranged widely [Markowitz et al., 1993; Sundsfjord et al., 1994; Agostini et al., 1996; Azzi et al., 1996]. Immunosuppression causes reactivation, and virus is shed in the urine. Reactivation occurs after organ transplantation, immunosuppressive chemotherapy for malignancy, and during pregnancy, old age, and AIDS [Reese et al., 1975; Coleman et al., 1980; Andrews et al., 1983, 1988; Gardner et al., 1984; Arthur and Shah, 1989; Kitamura et al., 1990; Flaegstad et al., 1991; Sundsfjord et al., 1994; Shah, 1996]. The frequency of excretion of JCV does not increase significantly in HIV-infected patients, whereas both frequency and level of shedding of BKV does increase [Markowitz et al., 1993; Sundsfjord et al., 1994; Azzi et al., 1996]. This reactivation is not reflected in serology, however, as no correlation was observed between serum BKV antibodies and degree of immunodeficiency in HIV-1–infected people [Sundsfjord et al., 1994]. BKV reactivation has been suggested to be the cause of hemorrhagic cystitis in bone marrow transplant recipients and in HIV-infected patients, as well as ureteral obstruction in renal transplant recipients [Gardner et al., 1984; Gluck et al., 1994; Pappo et al., 1996; Shah, 1996]. JCV causes progressive multifocal leukoencephalopathy in immunocompromised individuals [Major et al., 1992; Shah, 1996], including in HIV-1–infected patients [Berger and Concha, 1995; Hair et al., 1992].

A retrospective serological survey such as this one cannot provide definitive proof that the neutralizing activities detected were induced by SV40. However, several lines of evidence support the interpretation that the results are not explained solely by cross-reactivities of antibodies against a human polyomavirus. Importantly, we have detected SV40 DNA by polymerase chain reaction and sequence analysis in archival tissue specimens from several children we identified as having SV40 neutralizing antibody [unpublished data]. As 70 to 80% of adults have antibodies to BKV and to JCV [Frisque, 1994; Shah, 1996], higher frequencies of apparent reactivity to SV40 should be observed if cross-reactions were being detected, especially in individuals with very high titers to BKV. In addition, if that were the case, the post-1962 adult age group should not differ in antibody reactivity from the older age groups, which it did.

Neutralization tests based on abrogation of virus infectivity are a highly specific measure of virus antibodies [Hsiung et al., 1994]. SV40 neutralization assays are not influenced by some known antigenic cross-reactivities among polyomaviruses which would be measured in other types of assays. There are shared epitopes on the T-antigens of SV40, BKV, and JCV [Dougherty, 1976; Beth et al., 1977; Ball et al., 1984], but as those are replication proteins that are not part of the virions, the cross-reactivities have no influence on assays of neutralizing activity. There is a genus-

specific determinant shared by all polyomaviruses on the major capsid protein VP1 that is buried inside the virus particle. Antibodies to this epitope are produced in response to disrupted virus particles or to purified VP1 polypeptides, but not to intact virus or during natural infections [Shah et al., 1977]. Such antibodies can react with virus-infected cells but are not neutralizing, so even if present, such antibodies would not score in the SV40 plaque reduction assay. High-titer hyperimmune rabbit sera, raised by multiple injections of purified virus, sometimes display low-level cross-reactivities with other polyomaviruses (at dilutions much lower than the titer against the homologous virus) [Penney and Narayan, 1973; Takemoto and Mullarkey, 1973]. We found that hyperimmune antiserum against BKV failed to neutralize SV40, whereas hyperimmune antiserum against SV40 had weak activity against BKV. Acute sera, obtained following a single injection of virus, are lower in titer and specific for the homologous virus [Penney and Narayan, 1973], and it is likely that a natural infection, which elicits low levels of homologous antibody, would be more similar to an acute serum. A previous study showed that volunteers who had been exposed to a virus vaccine contaminated with SV40 did not show any changes in antibody to BKV, although they developed neutralizing antibody to SV40 ranging in titer from 1:5 to 1:80 [Brown and Morris, 1976]. Further, we showed here that there was no correlation between the presence of SV40 neutralizing antibody and the titer of BKV neutralizing antibody in a given human serum, an observation we have confirmed in a preliminary study of children's sera [unpublished data]. These data indicate that SV40 reactivity detected in our neutralization test was not simply a reflection of high levels of BKV antibody. A limitation of this study was that sera could not be tested for JCV neutralizing antibody, as there is no readily available cell culture system in which to propagate JCV and quantitate neutralization of JCV infectivity. Finally, we have not excluded the theoretical possibility that an unrecognized human virus is responsible for the induction of cross-reacting antibodies detected in the SV40 plaque reduction assay.

The means of transmission of SV40 infections in humans remains to be established. However, based on what is known about natural infections of rhesus monkeys by SV40 [Shah and Nathanson, 1976], it is reasonable to predict that SV40 in humans would resemble the human polyomaviruses (i.e., shed in the urine and infect via the oral route or respiratory tract). The fact that there was no significant difference in the SV40 seropositivity rates of HIV-infected and HIV-negative individuals in our study indicates that risk factors leading to HIV infection do not result in increased rates of SV40 infection. Future studies should address the age at which SV40 infections are normally acquired (in childhood, in adolescence, or as adults).

We conclude that there is credible evidence of SV40 infection in about 10% of the adult males in our study population. SV40 seroprevalence was not affected by HIV infection status or race/ethnicity background. A more comprehensive study will be necessary to establish definitively the frequency of infection in different age groups. This study suggests that SV40 infections of humans do occur and that there is the theoretical possibility that SV40 might contribute to tumor development or other disease in HIV-infected persons or other immunocompromised patients.

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